1 Impact of Magnetic assisted Freezing in the physicochemical and
2 functional properties of egg components. Part 1: Egg White

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ABSTRACT

Effects of freezing assisted with magnetic fields (MFs) in a Cell Alive System (CAS) commercial unit at -50°C , with a static MF only (0% CAS) and with a static MF plus oscillating MF (10% CAS) on egg white was investigated. Egg white (W) was from commercial eggs from three strains of laying hens (two of them living in fortified cages and the third one in liberty at outdoor). The main aim was to follow the protein thermal denaturation involved in W processing. Results showed that freezing treatment under magnetic fields was the factor with the highest influence. Thermal denaturation enthalpy of W was markedly affected ($\sim 50\%$ total protein in comparison with fresh sample) but similar for both MFs processes (no practical differences relative to conventional freezing). Rheology and free Sulfhydryl content were studied complementarily to Differential Scanning Calorimetry (DSC) data. Apart to proximate composition and pH, some important functional properties as Foaming parameters and Texture of W gel, were also determined to track their changes along MFs processing.

Key words

Egg white; Magnetic assisted freezing; Physicochemical properties; Functional properties; Protein denaturation; Rheological behavior.

1. Introduction

Hens' eggs have been used as a food by human beings since antiquity and there is no other single food of animal origin that is eaten by so many people all over the world and none is served in such a variety of ways. Eggs are generally considered as tasty, wholesome, and nutritious food. Their protein value is high while their calories and fat content are in moderation. In addition, eggs are easy to digest. They are very good potential sources of fresh materials for health-promoting derivatives, the so called functional foods, as well as an ingredient for the food industry in general. Also they are suitable for nutritional improvement (fortification) of several kinds of foods since they have four major nutritional components: proteins, lipids, all necessary vitamins (except vitamin C), and minerals. Egg products are thus used in

the food industry because of their nutritional value and organoleptic characteristics, but also and widely by their functional properties. Some of these functional properties include, foaming, thickening, binding, and emulsifying; in addition, the coloring ability and aroma of egg should also be mentioned. Egg products are thus used in the food industry because of their nutritional value and organoleptic characteristics, but also and widely by their functional properties as appearing when a controlled heating in dry state is applied (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989) or when combining preheating and freezing to determine the characteristics of gel properties (Xu, Shimoyamada, & Watanabe, 1997).

All eggs have two main edible parts: White (or Albumen), (W), and Yolk, (Y). The main proteins of W are: Ovalbumin, the predominant protein (54%), is a monomeric phosphoglycoprotein of 45 kDa containing 1 disulfide bond and 4 sulphydryl groups buried within its core that become exposed upon processing to form intermolecular linking yielding gel structures. Ovalbumin is the main responsible for the W functionality (Arzeni, Pérez, & Pilosof, 2012). Ovotransferrin (or Conalbumin) is a glycoprotein responsible for the transfer of ferric ions from the hen's oviduct to the developing embryo; Ovomucoid is a glycoprotein that shows a trypsin inhibitory activity and due to this enzyme inhibition function is very stable against digestive enzymes and high temperatures; Ovomucin is a sulphated glycoprotein with a highly viscous and gel-like structure, its content in thick egg white is four times higher than in thin white. W has no specific macrostructure but three layers can be distinguished in it: an inner layer of thin white, a layer of thick white, richer in ovomucin (the chalaziferous tissue that is usually removed for egg white study), and an outer layer of thin white. It is considered a colloidal suspension of different protein constituents. Ovomucin is thought to be the only responsible for the gel-like properties of fresh thick egg albumen; it forms an interlinking network of mucin fibrils that prevents bacterial movement within the egg white (Alleoni, 2006). Albumen alone is abundantly used (after globulins) in industrial applications due to its excellent foaming properties of rapidly adsorbing on the air-liquid interface during whipping or bubbling, forming a cohesive viscoelastic film by intermolecular interactions induced by the different charge characteristics of the constituent proteins (Damodaran, Anand, & Razumovsky, 1998).

Egg components are susceptible to be treated by the new technological processes for the inactivation of microorganisms/enzymes to ensure a safe consume at short time or to extend their self-life at long storage periods. Among these treatments we are particularly interested in Magnetic Assisted Freezing (MFs), a novel method that has not been applied to egg products so far as we know. MFs combines the application of a variety of magnetic fields with temperatures below the freezing point of the samples; its commercial development is giving promising results (Xanthakis, Havet, Chevallier, Abadie, & Le-Bail, 2013; Xanthakis, Le-Bail, & Ramaswamy, 2014), although the number of studies reported in the literature still is very scarce and to date, clear evidence of the promised effects has not yet been found. Notwithstanding, interesting contributions on the MFs state of the art have been recently reported (Otero, Rodríguez, Pérez-Mateos, & Sanz, 2016), where its fundamentals and application to biological systems were largely considered. MFs applications to garlic bulks (James, Reitz, & James, 2015) and crab sticks (Otero, Pérez-Mateos, Rodríguez, & Sanz, 2017) have been dealt with. Additionally, MFs has an important local interest because this emerging technology is currently in a phase of commercial introduction in many countries, including Spain.

The main goal of present work is to study the effect of MFs treatments, performed in a commercial unit, on several physicochemical and functional properties of Egg White (W) from three classes of commercial eggs laid by three types of hens living in different conditions. Among the physical properties to be studied a major one is the thermal denaturation of respective proteins involved in MFs treatments by Differential Scanning Calorimetry (DSC), complemented by Textural and Rheological analysis, as well as by chemical determinations of Free sulfhydryl (SH) content. Interesting of remembering is that rheological properties are among the most important characteristics of food products determining the functional properties, product development strategies and shelf-life of the products, and are useful for the design of processing equipment such as pipes, pumps, mixers, extruders, homogenizers, and heat exchangers (Steffe, 1996; Singh, Sharma, Premi, & Kumari, 2014).

The collected data could help understanding the changes that this new freezing technology may induce in the respective physicochemical or functional properties like foaming or gelling. So that, concerning egg components treatment, we could be able to present a general evaluation of the MFs performance in comparison with respective untreated Fresh egg components.

2. Materials and methods

2.1. Sample preparation

Three different brown egg classes, laid within 7 days, all from *Gallus Gallus* hen flocks fed compound-feed were obtained from a local producer. Three laying hens' strains corresponded to Lohmann Brown (A), Hn Brown (B), and Isa Brown (C) respectively. While A and B hens lived kept in fortified/enriched cages, C hens remained free at outdoor eating grass in addition.

Fresh egg white (W) was manually separated from each egg, and the chalazae and the rest of spots and defects were removed (Manzocco, Panozzo, & Nicoli, 2013; Song, Kim, Choe, Jung, Kim, Kim, & Jo, 2009). Not less than 20 eggs from each strain were broken for the preparation of each W sample batch. The resulting W was gently homogenized (so as not to produce foam and to avoid/minimize water losses) by a magnetic stirrer (VWR 700 Advance Series) at 20 °C for 1h before sampling, and then immediately processed.

2.2. Composition and pH

Compositional data were determined by triplicate according to AOAC methods (1984): Water content was evaluated by desiccation of ~5 g using an air-forced furnace Selecta 2000367 (JP SELECTA, SA, Spain) at 105 °C until a constant weight was reached. Fat was determined by the Soxhlet method with a Soxtec system Tecator AB S-263 21 (Höganäs, Sweden). Protein was determined by the Dumas method with an analyzer LECO TruMac (Leco Corporation, St. Joseph, MI, USA). Ash content was determined with an oven Select Horn (JP SELECTA, SA, Spain) working at 550 °C for 5 h.

The pH was determined at room temperature with a pH-meter (Crison, pH Burette 24 1S) by triplicate.

2.3. MFs treatments

A freezer (ABI, Corporation Ltd., Chiba, Japan) equipped with both static and oscillating magnetic fields (OMF) generators (Cell Alive System – CAS) was used. Details about its characterization are given in (Otero, Pérez-Mateos, Rodríguez, & Sanz, 2017). Among possibilities of the freezer, two of them corresponding to the minimum and to the maximum magnetic intensity field were chosen, i.e., one with

only static MF (0% CAS) and the other with the static MF plus OMF (10% CAS). Magnetic field strength and frequency values measured at the center of the freezer were (0.14 mT, 0 Hz) and (1.66 mT, 6Hz) for 0% CAS and 10% CAS respectively. The temperature of the freezer was programmed to be -50°C . Air velocity measurements were carried out using an anemometer (VT100, Kimo S.A., Montpon, France) resulting to be 3.8 m/s. During each CAS freezing treatment, the evolution of the temperature versus time in the 100 mL sample was obtained by using a T1S-02-W10-PR10 fiber optic probe connected to an optic fiber temperature thermometer Reflex-4 (Neoptix, Canada) recording data each second. Both MFs treatments were applied for ~ 75 min processing time, until the sample achieved -40°C . Cooling rates ($^{\circ}\text{C}/\text{min}$) were very low, ranging $\sim 2.7 - 3.0$ in the initial step (before the phase change), and $\sim 1.3 - 2.3$ in the final step (after the phase change); with the higher and lower values corresponding to 0% CAS and 10% CAS respectively.

MFs at 0% CAS could be roughly considered as the corresponding control freezing treatment (Control) in practice since the static radiation used (0.14 mT) was close to that naturally received at Madrid and periphery. In fact, it was 0.044 mT according to the Observatorio San Pablo de los Montes, Toledo (Instituto Geográfico Nacional). The estimated value by the Magnetic Field Calculators from the National Center for Environmental Information (NCEI, USA) using the Madrid's geographic data of Latitude ($40^{\circ} 26' 32''$ N), Longitude ($3^{\circ} 41' 27''$ W) and Elevation (0.655 km) was 0.04478 mT, in agreement with the determined one. Interesting to remark is that Conventional Freezing may not be chosen as Control, because this process is usually performed at selected temperatures but at very high cooling rates. They are not accessible in MF processing due to its intrinsic, very low cooling rates, as shown above. It is worth to mention the interesting considerations done by Wowk (2012) in his Letter to the Editor concerning a proper selection of Control in CAS freezer findings.

In the case of DSC and Rheology, the procedure used for analysis was: Fresh W (WA, WB, WC) samples were prepared in the morning and subsequently subjected to MFs freezing treatment; the frozen samples were then kept in the refrigerator ($2 - 4^{\circ}\text{C}$) overnight for thawing and then immediately encapsulated and DSC run or rheological measurement, keeping the operating conditions always at temperatures

lower than 20 °C, to avoid undesirable protein denaturation. However, and just in case this way were not suitable to be managed in most of the physicochemical determinations, a total of 72 W individual 100 mL portions for each CAS condition from the above referred W (WA, WB, WC) sample batch, were deep-frozen at -80 °C with a Forma -86C ULT Freezer (Thermo Fisher Scientific, Waltham, MA, U.S.A) at a cooling rate of 1 °C/min. Successively, samples were thawed for two hours at room temperature for daily analytical use. This process did not endure more than twenty days. This frozen storage was chosen in order to highly reduce food degradation reactions kinetics, as expressed by the Arrhenius equation in that the lower the storage temperature the minor the kinetic constant. In addition, the high cooling rate used may not presumably modify but very slightly the original ice crystal size and the sample could remain very close to the original. Only determinations like Gel firmness and Foam properties were affected and, fortunately their results (see corresponding Results headings) seemed not to observe inconsistent values relative to the general study.

The rationale for not subjecting DSC and Rheological samples to above recrystallization is: The phase diagram of water as a function of temperature has been well established at atmospheric, hypo- and hyper-baric conditions, and it is known how the ice crystals-liquid water coexistence may evolve due to crystallization increments produced subsequently to temperature decrements. The process not only implies an increase in the ice crystals number but also a re-crystallization phenomena by which the larger crystals enlarge in size, with the ice volume fraction increment, to the expense of the smaller ones. It leads to higher salts concentrations, i.e., ionic strength augmentation in the persisting liquid water which, in turn, may induce protein denaturation in the aqueous protein matrix. Concerning this, Martino & Zaritzky (1989) performed a nice study of ice recrystallization in a model system (0.28 N NaCl aqueous solution) subjected to freezing and frozen storage at several temperatures. In their experience this solution behaves similar to meat. The argentinean authors also studied the ice recrystallization phenomena on a semitendinous beef muscle subjected to same conditions and, in fact, the meat behavior was satisfactorily modelled. They finally concluded reporting miofibrillar protein denaturation effects in the muscle meat.

Although current egg proteins are globular, they underwent also thermal denaturation by freezing.

2.4. Free sulfhydryl (SH) content

The content of free sulfhydryl moiety of W samples was determined using the Ellman's reagent (5', 5-dithiobis 2-nitrobenzoic, DTNB) (Sigma Aldrich, 500mg), as reported by Beveridge, Toma, & Nakai (1974), i.e., about 1.5 g sample were solubilized in 10 mL 0.086 M Tris buffer containing 0.09 M glycine plus 4 mM EDTA (10.4 g Tris base, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0). Each of six aliquots (0.1 mL) of sample solution was then mixed with 2.9 mL 0.5% (w/v) SDS in the same buffer plus 20 μ L Ellman's reagent (4 mg/mL in the same buffer). After 15 min reaction at room temperature, the sample was centrifuged for 20 min at 19900 $\times$ g, and the supernatant used for the absorbance determination at 412 nm in a spectrophotometer Thermo Scientific, Genesys 10S-UV-Vis, using the buffer as a blank. The SH content was calculated by, $SH = 73.53 \times A_{412} \times D/C$, where A_{412} is the absorbance of the sample, D is the corresponding dilution factor (30.2), and C is the sample concentration in mg/mL on dry bases. The coefficient 75.53 is derived from the molar absorptivity, or molar extinction coefficient (1.36×10^4 cm/M) of the Ellman reagent (Ellman, 1959). Measurements, expressed in μ M/g, on the six aliquots were averaged.

2.5. Foam ability and stability

Foaming properties were investigated based on the method of Manzocco, Panozzo, & Nicoli (2013), with some modification. Foams were processed by whipping 10 mL of fresh W for 3 min at 20 °C in a 50 mL cylinder by a high speed mixer (IKA-WERKE T25 BASIC) operating at 11000 rpm. The volumes of the foam and the drained liquid were assessed just after whipping and during holding up for 30 min at 20 °C. Foam ability (FA, %) and stability (FS, %) were calculated as follows,

$$FA = (V_f - V_0)/V_0 \times 100$$

$$FS = V_{f_{30}}/V_0 \times 100$$

Where V_f is the foam volume, V_0 is the initial volume of W, and $V_{f_{30}}$ is the foam volume after 30 min observation. Determinations were performed in sextuplicate.

2.6. Gel firmness

10 g W samples were transferred to 20 mL capacity glass vials, which were then closed with rubber stoppers. Samples were heated in a water bath at 90 °C for 5 min. Then, tubes were immediately cooled with ice-water and kept at 4 °C overnight. Samples were then removed from the vials and cut into two pieces (2 cm diam. and 1.5 cm height), tempered at 20 °C, and thus punctured with a flat-ended cylindrical stainless steel probe of 6 mm diam. at the speed of 0.5 mm/s, using a 5 kg load cell on a Texture Analyser TA-XTplus (Stable Micro System Ltd., Surrey, UK) controlled by software (Texture Exponent v6.1.5.0). The force (N) to puncture 0.4 cm thermo-irreversible gel was recorded at least 10 times and the average reported.

2.7. Rheological measurements

Oscillatory shear measurements were conducted using a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK), with cone-plate geometry (cone angle 1°, gap 0.03 mm). Temperature was controlled at 25 °C. Frequency sweep tests were carried out at the frequency range 0.1–10 Hz with a strain of 30%, selected from the linear viscoelastic region (LVE), to evaluate the dynamic rheological properties including storage modulus (G' , Pa) and loss modulus (G'' , Pa). The exponent n was calculated from the power law equations, $G' = G_0' \times \omega^n$ and $G'' = G_0'' \times \omega^n$, where ω is the angular frequency (Hz), and G_0' and G_0'' are the storage and loss moduli respectively, determined at 1 Hz. Measurements were at least by triplicate.

2.8. Differential scanning calorimetry (DSC)

A model TA-Q1000 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA) was used to determine temperatures and enthalpies of protein denaturation in both fresh and processed samples. W samples were diluted with an aqueous solution of NaCl 1% (w/v) solution, so as to obtain a suspension containing 80% (w/w) pure W and 20% NaCl 1% (w/v), that were gently stirred manually for homogenization, as recommended by Guilmineau & Kulozik (2007) for other kind of application. This dilution was aimed to ensure protein denaturation without limitations by insufficient water content, the viscosity being then reduced, thus facilitating also an easier sampling; additionally, the electrolyte presence may help to minimize the protein insolubility which could be derived from the diluting process. Aliquots of about

8 – 10 mg (± 0.002 mg) were weighed out using a model ME235S electronic balance (Sartorius, Goettingen, Germany), tightly encapsulated in hermetic aluminum pans and scanned under dry nitrogen (50 mL/min) purge. An empty capsule was used as reference. Samples were heated from 5 to 100 °C at 10 °C/min and, after cooling down to 5 °C at 10 °C/min, a second scan in the same conditions was occasionally run to check for reversible effects. Peak temperatures (T_{peak} , °C) and transition enthalpies (ΔH , J/g_{dm}) by linear baseline integration were calculated and normalized to dry matter content by encapsulated-sample desiccation (a pin-hole was practiced on the top of the capsule cap and then subjected in a regulated oven to 90 °C and reduced pressure, 720 mm Hg, overnight). Samples were analyzed by quintuplicate.

2.12. Statistical analysis

One-way analysis of variance (UNIANOVA) was carried out (IBM SPSS Statistics v22.0, Armonk, NY, Inc.) to determine the influence of the source of variation due to the freezing treatment and the strains of laying hens by taken into account dependent variables with high loading F values and high correlation coefficients ($r^2 \geq 0.80$). The difference of means between pairs was resolved using confidence intervals with the Bonferroni or Tamhane tests depending on the variance homogeneity. Level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Composition

Table 1 shows the macro-constituents distribution in native W samples according to the three egg classes (A, B, C). WA and WB did not present significant differences concerning water, protein and ash contents, while WC exhibited significantly different results in water (lower) and protein (higher).

3.2. MFs freezing diagrams

Figure 1 records the Time-Temperature typical diagrams of MFs treatment of W samples (no relevant differences among classes WA, WB, and WC were observed) at both processing conditions of 0% CAS and 10% CAS. W samples showed similar profiles in both MFs processes: a considerable freezing plateau (shorter in 0% CAS) with some slope and asymptotic-like declining (more pronounced in 10% CAS) that

makes end point difficult to be appreciated, presumably due to inaccuracy in probe positioning at the sample holder center. MFs cooling curves morphologies were quite similar to those of conventional freezing processes. Ice crystallization was apparently bigger in 10% CAS, but variations among different samples (due to above comments) did not confer any significance to it.

3.3. pH

Among others, pH is an essential attribute to achieve adequate functional properties of egg white and yolk. So the change of pH during the handling, preparation, treatment and storage, is more important and can be used to determine the egg freshness and its functionality before application.

The influence of the egg lied strains on the pH of W (WA, WB, WC) samples were not significantly different, so that the below data corresponded to the W mean values for the three strains,

W: Fresh, 9.17 ± 0.11^a ; 0 % CAS, 9.45 ± 0.50^b ; 10 % CAS, 9.41 ± 0.03^b

where different lowercase exponents indicate significantly differences ($p < 0.05$) among samples. MFs samples were somewhat, significantly higher in pH relative to Fresh but not significantly different between 0% CAS and 10% CAS. It apparently seemed that pH increments in W processed samples by MFs with respect to corresponding Fresh sample could be initially due to the thermal change of state involved. However, Heath (1977) and Caner & Cansiz, (2008) have reported that fresh albumen contains a high concentration of carbon dioxide that during the storage starts to release and leading to a pH increase. Therefore, current W processed values were relatively higher as corresponding to the additional aging undergone along MFs treatments. De Souza & Fernández (2011) reported similar values for differently treated W samples (homogenized, pasteurized, UV static, UV dynamic) that were related also to the CO₂ exchanges involved.

3.4. SH

Sulfhydryl (SH) groups influence significantly the functional properties of food proteins and play an important role in the formation of specific structures such as protein gels and foams. Figure 2 shows SH results determined in samples W at 0%

CAS and 10% CAS vs. Fresh, untreated samples. SH data on Fresh W samples are mainly due to ovalbumin containing four sulphydryl moieties; they were somewhat lower than the reported value (50.7 $\mu\text{M/g}$) by Beveridge, Toma, & Nakai (1974), or the $58.5 \pm 3.0 \mu\text{M/g}$ from Van der Plancken, Van Loey, & Hendrickx (2007). Badr (2006) employed the same methodology as the other authors and reported 43.07 g/kg (dry bases), in the line of resting results, for W untreated sample in a study of gamma radiation application and cold storage with a microbiological aim of egg preservation. Arzeni, Pérez, & Pilosof (2012) dealing with high intensity ultrasounds application to 10% aqueous solutions of dried egg white, reported higher values ($50.53 \pm 0.61 \mu\text{M/g}$) as due to an increase of surface hydrophobicity on processed samples. Wu, Zhao, Yang, & Chen (2014) studied the effect of pulse electric fields on the stability of egg white solutions, reporting $\sim 41 \mu\text{M/g}$ for untreated samples, that increased along processing and led to protein aggregation.

It appeared that current W samples presented similar values in both freezing cases, with no significant differences between treatments ($r^2 = 0.26$) and among strains. MFs yielded not significantly higher SH amounts in 0% CAS and 10% CAS relative to Fresh sample, which may indicate minor conformational changes in the processed W proteins. Once dealing with average SH values for the three strains in the three cases: 43.74 (Fresh), 43.55 (0% CAS), and 45.46 (10% CAS), they were similar for untreated and for both MFs processed samples.

Comparisons of current MF data were not available because no prior studies have been reported in the literature.

3.5. Foam Ability and Stability

Foaming or whipping, i.e., the ability to form stable foams with air, is an important functionality of proteins in several products (cakes, divinity-type confections, candy, meringue, soufflés, various whipped toppings, icings, fudges, nougats, etc.). It depends on a critical balance between proteins ability to engage in limited intermolecular cohesion required to form a stable elastic film, and the tendency to self-associate excessively, which would result in aggregation and breakdown of foam. Proteins must have the ability to adsorb rapidly at the air-water interface during whipping or bubbling; secondly, they had to undergo a rapid conformational change and rearrangement at the interface; finally, it must provide the possibility to form a cohesive viscoelastic film via intermolecular interactions (Mine, 1995; Du,

Prokop, & Tanner, 2002; Lomakina & Mikova, 2006), which is facilitated by some degree of surface denaturation. This accentuates protein-protein interaction and enhances cohesive forces between the proteins in the film capsule (Kinsella & Melachouris, 1976; Van der Plancken, Van Loey, & Hendrickx, 2007). However, complete denaturation is undesirable, because it results in membrane fragility and foam collapse. Foam ability (FA), or overrun, of W (WA, WB, WC) samples is shown in Figure 3(Top). Freezing by both MFs treatments considerably reduced foam ability of Fresh W but, no significant differences were detected between the two freezing treatments ($r^2 = 0.68$). Arzeni, Pérez, & Pilosof (2012) in the already commented study on 10% solutions of dried egg white, gave 205 ± 21 (%) for foam overrun, in line with current data. Figure 3(Bottom) shows the corresponding Foam Stability (FS) data. Results for the three W classes did not observe significant differences in the two freezing treatments ($r^2 = 0.27$). It seemed a reasonable consequence of the fact that protein denaturation is, by far, much more dependent on the frozen temperature and time of storage than on the freezing process itself (Careche, del Mazo, & Fernández-Martín, 2002). Data to be directly compared with ours were not found previously reported, as they rather dealt with kinetics.

3.6. Gel firmness

Figure 4 shows the evaluation of W thermal gel firmness (N) results in Fresh and MFs frozen samples at 0% CAS and 10% CAS. Fresh WA and WB behaved similar in heat induced gelling but WC produced significantly stiffer gels, as corresponding to its significantly higher protein content (Table 1). MFs treatments yielded close results between 0% CAS and 10% CAS conditions. Those, in turn, were similar to Fresh results, which pointed to predominant thermal effects over magnetic ones in MFs processing. The great data scattering led to high standard deviation values which could partially mask differences among results. Woodward & Cottrell (1986) studied texture behavior of egg white gels as influenced by different treatments (freeze-dried then reconstituted at several protein concentration, temperature and heating time, pH, NaCl addition) and reported compression hardness values ranging ~1 – 3 kg. Kitabatake, Shimizu, & Doi (1988) studied transparent thermal gels behavior from dialyzed egg white as a function of pH and ionic strength; hardness values ~350 g (~36 kg/cm²) were reported by compression of samples without NaCl added, which is higher, as expected, but with the same magnitude order than current

ones. Kitabatake, Tani, & Doi (1989) studied dialyzed ovalalbumin (60 and 70 mg/mL) gels, either transparent or turbid, as a function of processing parameters (temperature/time) and gel preparation (pH, ionic strength), reporting compression hardness values that may be extrapolated to a range averaging $\sim 3.5 \times 10^4 \cdot \text{dyne/cm}^2$, equivalent to $\sim 35 \text{ N/cm}^2$, which is not comparable with current ones belonging to a much lower protein concentration ($\sim 13.5 \text{ mg/mL}$). Matsudomi, Tomonobu, Moriyoshi, & Hasegawa (1997) reported compression hardness in 10% (w/v) egg white protein of $\sim 28 \text{ g}$, it being not able to be converted into current units. Houska, Kýhos, Novotna, Landfeld, & Strohaln (2004) reported gel strength data (p/cm^2) of native egg white of brown and white eggs from various farms, subjected to different conditions of pH, protein content and egg aging. It led to a range of values that comprises those currently ($\sim 360 \text{ p/cm}^2$) reported. No prior data have been found on MFs treated W samples for comparison.

3.7. Rheology

Dynamic rheological properties are among the most important characteristics of food products determining their functional properties, product development strategies and shelf-life, and are useful for the design of processing equipment such as pipes, pumps, mixers, extruders, homogenizers, or heat exchangers (Steffe, 1996; Singh et al., 2014). Oscillatory testing is the most common dynamic method to study the viscoelastic behavior of food products. It describes material properties in terms of storage modulus G' (elastic behavior) and loss modulus G'' (viscous behavior). The effects of MFs processing at 0% CAS and 10% CAS on rheological properties of egg white W from three different hen strains (WA, WB, WC) were evaluated and compared with Fresh samples. For more detailed comparison, G' and G'' results of frequency sweep test from 0.1 to 10 Hz measured at 25°C are presented in Figure 5(Top) for W (WA, WB, WC). Regardless the hen strain or the MFs treatment, the mechanical spectra revealed a similar evolution of G' and G'' as a function of frequency: either untreated (Fresh) or MFs processed (0% CAS and 10% CAS) demonstrated that the two moduli followed parallel patterns with $G' > G''$, thus indicating a predominantly elastic behavior (concentrated viscous solution similar to that typically found in polymeric dispersions) in all egg white samples over the whole frequency range. Mechanical spectra of G' did not fit properly the power law model ($r^2 < 0.85$ in all cases), therefore no conclusive information from the comparison of

the exponent n' values could be provided. Anyway, differences among samples were found to be very scarce. As already commented, the gel-like properties of raw egg albumen is mainly attributed to ovomucin crosslinking (Alleoni, 2006). Different interactions, such as hydrogen and disulfide bonds, hydrophobic and ionic interactions are responsible for this internetwork, especially in thick white. Statistical data of G' and G'' determined at 1 Hz in W (WA, WB, WC) are shown in Figure 5(Middle) and 5(Bottom), respectively. G_1' and G_1'' moduli were not significantly different among Fresh and MFs processed samples ($r^2 = 0.18$ and 0.23 respectively). MFs did not cause substantial protein alteration so as to modify significantly the native viscoelastic behavior of egg white.

3.8. DSC

Figure 6(Top) shows typical normalized DSC traces of Egg White (WA) of untreated (Fresh, solid line) and MFs processed samples at $-50\text{ }^{\circ}\text{C}$ and 0% CAS (Dash line) or 10% CAS (Dot line). Due to the usual pH values of the samples of ~ 9 , denaturation of conalbumin and lysozyme appeared entirely overlapped in a single zone (Donovan, Mapes, Davis, & Garibaldi, 1975) with a characteristic $T \sim 67.5\text{ }^{\circ}\text{C}$. The main peak at $\sim 81.7\text{ }^{\circ}\text{C}$ corresponded to ovalbumin, the major W protein, which was followed by a minor one at $\sim 87.1\text{ }^{\circ}\text{C}$ due to the prior presence of S-albumin in the samples.

The three samples exhibited similar DSC profiles with partial areas distribution of $\sim 33/67$ at $T = 75\text{ }^{\circ}\text{C}$, this pointing to similar denaturation processes with progressive denaturation of each one and all the proteins. MFs treated samples showed highly reduced areas under their curves relative to Fresh sample. Protein denaturation was easily visible in all cases because the clouds appearance in the processed samples.

Thermal denaturation enthalpy data are graphically shown in Figure 6(Top) as affected by the three hen strains (WA, WB, WC), with their statistical evaluation. Normalized ΔH (J/g_{dm}) (nearly equivalent to ($\text{J/g}_{\text{protein}}$) as ovoalbumen is over 90 % w/w protein rich) values were considerable and significantly larger in Fresh ($r^2 = 0.92$) than in MFs frozen samples where, despite the somewhat higher denaturation in 10% CAS depicted in Figure 6(Top), no significant differences between both 0% CAS and 10% CAS were found. This seemed pointing to a thermal nature of the

predominant cause of protein denaturation against magnetic effects in the MFs processing. No significant differences among strains were observed in Fresh and in both frozen samples. Thermal data (both temperatures and enthalpies) for W Fresh sample were somewhat greater than those reported in the literature (Donovan, Mapes, Davis, & Garibaldi, 1975; Barbut & Findlay, 1990; Ferreira, Hofer, & Raemy, 1997; Arzeni, Pérez, & Pilosof, 2012; Sharma, 2012; Souza & Fernández, 2013) because all these were determined at pH 7, while current determinations were performed at pH 9. Ibanough & Erçelebi (2007) reported 23.3 ± 0.6 J/g for a commercial W dried powder which was dissolved in phosphate buffer (pH 7) containing 0.17M NaCl. Several other studies have also appeared reporting consistent DSC traces of W with peak temperatures but without any enthalpy data associated, or by separated peak integration without integral enthalpy data (Sing & Ramaswamy, 2014), even with (in our opinion) apparently erroneous baselines construction yielding undervalued integration results (Németh, Harváth, Drobecz, Friedrich, Pásztor-Huszár, & Balla, 2010). DSC data were consistent with SH increasing and FA decreasing determinations, as expected. No prior data on MFs freezing was found for comparison.

Conclusions

Fresh white egg samples were highly affected by MFs treatments as concerning their protein denaturation enthalpies. The other physicochemical and functional properties studied like, SH, FA and FS, as well as Gel Firmness, G' and G'' moduli were, in general, slightly (SH increasing, FA decreasing) and majorly not significantly affected by MFs treatments, probably masked by the large variations they observed. With regard to hen strains, they did not differential- but exceptionally influenced the physical or functional behaviors of W samples both untreated and processed samples. Thawed MFs frozen samples exhibited highly denatured/aggregate egg proteins with reduction in their functional properties. Concerning egg white processing, it can be concluded that oscillating MF treatment (10% CAS) did not present any advantage over respective freezing control at the same processing conditions (Control, 0% CAS).

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Figure legends

Figure 1. Temperature (°C) vs. time (min) typical diagrams of Egg White (W), Green lines; under MFs treated at -50 °C and 0% CAS (Dash lines) or 10% CAS (Solid lines). Processing temperatures, Blue lines.

Figure 2. Mean values $\pm$ standard deviations of Free Sulfhydryl Content (SH, $\mu\text{M/g}$) of Egg White (W): WA, ; WB, ; WC, ; as a function of processing: Untreated, Fresh; MFs treated at -50 °C and 0% CAS or 10% CAS. Different lowercase letters indicate significant ($p < 0.05$) differences among treatments within a strain. Different uppercase letters indicate significant ($p < 0.05$) differences among strains within a treatment.

Figure 3. Mean values $\pm$ standard deviations of **Top**) Foam Ability (FA, %), and, **Bottom**) Foam stability (FS, %), of Egg White (W): WA, ; WB, ; WC, ; as a function of processing: Untreated, Fresh; MFs treated at -50 °C and 0% CAS or 10% CAS. Different lowercase letters indicate significant ($p < 0.05$) differences among

treatments within a strain. Different uppercase letters indicate significant ($p < 0.05$) differences among strains within a treatment.

Figure 4. Mean values $\pm$ standard deviations of Gel firmness (N) of Egg White (W): WA, ; WB, ; WC, as a function of processing: Untreated, Fresh; MFs treated at -50°C and 0% CAS or 10% CAS. Different lowercase letters indicate significant ($P < 0.05$) differences among treatments within a strain. Different uppercase letters indicate significant ($P < 0.05$) differences among strains within a treatment.

Figure 5. Top) Frequency sweep test (0.1 – 10 Hz) of Egg White (W): WA, Triangles; WB, Squares; WC, Circles; for Storage Module (G' , Pa), Open Symbols; and for Loss Module (G'' , Pa), Solid Symbols. **Middle)** Mean values $\pm$ standard deviations of Storage Module (G'_1 , Pa) at 1 Hz; and, **Bottom)** Mean values $\pm$ standard deviations of Loss Module (G''_1 , Pa) at 1 Hz; of Egg White (W): WA, ; WB, ; WC, ; as a function of processing: Untreated, Fresh; MFs treated at -50°C and 0% CAS or 10% CAS.

Figure 6. Top) Normalized typical DSC traces (ΔH , J/g_{dm}) as a function of temperature ($^{\circ}\text{C}$) of Egg White (W). Samples: Fresh, Solid line; MFs treated at -50°C and 0% CAS (Dash line) or 10% CAS (Dot line). **Bottom)** Mean values $\pm$ standard deviations of Normalized Thermal Denaturation Enthalpy (ΔH , J/g_{dm}) of Egg White (W): WA, ; WB, ; WC, ; as a function of processing: Untreated, Fresh; MFs treated at -50°C and 0% CAS or 10% CAS. Different lowercase letters indicate significant ($p < 0.05$) differences among treatments within a strain. Different uppercase letters indicate significant ($p < 0.05$) differences among strains within a treatment.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Table 1. Mean values $\pm$ standard deviations (%) of main constituents (Water, Protein, Ash) of Egg White (W) from the three egg classes (A, B, C).

Classes	W		
	Water	Protein	Ash
A	88.17 $\pm$ 0.02 ^a	11.33 $\pm$ 0.11 ^a	0.76 $\pm$ 0.04 ^a
B	88.11 $\pm$ 0.09 ^a	11.47 $\pm$ 0.18 ^a	0.80 $\pm$ 0.05 ^a
C	86.73 $\pm$ 0.07 ^b	12.89 $\pm$ 0.27 ^b	0.73 $\pm$ 0.03 ^a

Different letters in the same column indicate significant ($p < 0.05$) differences.